DATA SHEET

Sapphire™ Baculovirus DNA

Insect cells and lytic baculoviruses provide a proven method for high-level expression of full-length mammalian proteins. Autpgrapha californica nuclear polyhedrosis virus (AcNPV) is used to infect cultured insect cells (e.g. Spodoptera frugiperda). Expression of the highly abundant polyhedrin gene is non-essential in tissue culture and its strong promoter can be used for the transcription of foreign genes. The polyhedrin promoter is maximally expressed very late stage in infection when the lytic virus kills the host cells, resulting in high levels of expression even for certain toxic proteins. In addition, many post-translational modifications similar to those in mammalian cells are made in insect cells and proteins able to be expressed in E. coli have been successfully expressed in the insect cell system.

Features

The Sapphire™ Baculovirus DNA offers the following advantages:

• Enhanced protein folding. The disulfide isomerases gene was inserted at the p10 locus of the virus to ensure proper protein disulfide bond formation.

• High recombination efficiency. The Sapphire™ baculoviral DNA contains a lethal deletion of ORF1629 which can only result in viable viral particles if rescued by homologous recombination with a polyhedrin promoter-based transfer vector. This design significantly reduces time and effort in plaque assays.

• High level expression. The p10 promoter is partially deactivated and the lytic p10 gene is deleted so that transcription levels are higher due to reduced interference and healthier insect cells.

Box 1 | Product Summary

Catalogue Number	ABP-BVD-10001
Component	One vial
Quantity	10 Rxns
Concentration	0.1µg/µl
Storage	Store at +4°C. Never freeze the viral DNA.
Stability	The Sapphire™ Baculovirus DNA is stable for six months when stored properly.

Storage Condition: +4°C

Recommended Storage of Recombinant Baculoviruses

Use the following procedure for long-term storage of virus stocks:

• Centrifuge viral stock at 4000 x g to remove cellular debris.

• If medium is serum-free, add serum to 10%.

• Store viral stocks at +4°C. However, virus titer may decrease 5- 10 fold during 6 months storage at 4ºC.

• Protect viral stocks from light to ensure maintenance of titer.

• For long-term storage (up two years), store small aliquots of virus at -80°C. Avoid repeated freezing and thawing.

Website: www.allelebiotech.com
Call: 1-800-991-RNAi/858-587-6645
(Pacific Time: 9:00AM~5:00PM)
Email: oligo@allelebiotech.com

Box 2 | Related Products

Product	Catalogue No.
Sapphire™ Baculovirus DNA and Transfection Kit	ABP-BVD-10002
Sapphire™ Insect Transfection Kit	ABP-BVD-10003
Sf9 Cells (in culture, 10^7 cells)	ABP-CEL-10002
Sf9 Cells (frozen, 10^7 cells)	ABP-CEL-10006
Sf21 Cells (in culture, 10^7 cells)	ABP-CEL-10003
Sf21 Cells (frozen, 10^7 cells)	ABP-CEL-10007
T.ni Cells (in culture, 10^7 cells)	ABP-CEL-10005
T.ni Cells (frozen, 10^7 cells)	ABP-CEL-10008
TNM-FH Insect Culture Medium	ABP-MED-10001
Serum Free Insect Culture Medium	ABP-MED-10002
Serum Free, Met Free Insect Culture Medium	ABP-MED-10003
Grace's Insect Culture Medium	ABP-MED-10004
pVL-1392-XylE Control Vector	ABP-BVP-10001
Insect Lysis Buffer 5X (10ml)	ABP-BUF-10010

Protocols

Co-transfection Using Sapphire™ Transfection Kit

1. Seed 1 x 10^6 Sf9 (Cat#: ABP-CEL-10001) or Sf21 (Cat#: ABP-CEL-10002) cells onto each 35 mm tissue culture plate. Gently tilt plates in a side to side and back-and forth pattern to seed the cells evenly and allow 5 to 10 minutes for cells to attach firmly.

Note: In addition to plates needed for co-transfection of genes of interest, prepare two additional plates, one negative control containing DNA Sapphire only and one positive control with Sapphire DNA and a suitable transfer plasmid containing marker (e.g., pVL1392 XylE: ABP-BVP-10001, or GFP).

2. Prepare DNA lipoplex transfection mixture as follows: In one tube, mix 0.5 ml Serum Free Insect Cell Medium and 3 ul Insect DNA shuttle transfection reagent. In another tube, add 1 ul Sapphire™ Baculovirus DNA, 1 µg transfer vector containing gene of interest, and 0.5 ml Serum Free Insect Cell Medium. Mix gently and combine content of both tubes into one and let sit at room temperature for 20 min to allow DNA lipoplexes to form.

3. Note: For cells in serum supplemented medium, rinse the monolayer 2X 1ml serum-free Medium. Take care not to disturb cell monolayer. Remove medium from cells and add DNA/transfection mixture from step 2.

4. Incubate 4 hrs at 27°C and remove/replace transfection mix with 2 ml of TNM-FH medium.

5. Continue incubation at 27°C and harvest medium containing recombinant baculovirus after 5 days. The expected titer of this initial P0 stock is ~ $1X10^6$ to $1X10^7$ pfu/ml.

Amplification of Recombinant Baculovirus

The recombinant baculovirus need to be amplified to obtain a higher titer stock solution after co-transfection. Freshly seeded cells should be infected at a multiplicity of infection (MOI) of <1.

1. Infect $5x10^6$ cells per 10 cm plate (approximately 60% confluent) with 500 ul of transfection supernatant in 15 ml of TNM-FH medium supplemented with 10% FBS.

2. Incubate cells at 27°C for 3 days before harvesting. At 24 hrs post infection virus-infected cells are visibly swollen with enlarged nucleus, which can be observed by light microscope. The medium will contain at > 10^7 pfu/ml.

3. Perform a second run of amplification as described in step 1. Two rounds of amplification usually give a virus titer of $2x10^8$ pfu/ml.

Titration of Amplified Virus Stocks

You must obtain a titer for a virus stock so that you can optimize subsequent infections to produce maximal yield of recombinant protein. Follow the end-point dilution assay outlined below to determine the titer of your virus stock.

1. Seed 2 X 10^5 Sf9 cells per well on a 12 well plate. Allow cells to attach firmly. Replace medium with fresh TMN-FH Medium containing 10% FBS (Cat. No. ABP-MED-10001).

2. Using the supernatant you wish to titer (usually obtained 5 days after the start of transfection), add 100µl, 10µl, 1µl and 0µl to different wells of the plate.

3. Incubate the cells at 27°C for three days. Examine the cells for signs of infection (i.e. enlarged nucleus and swollen cells).

4. A successful transfection should give you uniformely large infected cells in all of the wells except the 0µl wells (controls). If you transfected with Sapphire™ DNA alone, all the wells should look like the 0µl control well.

5. If only the 100µl and 10µl well seems to have infected cells and the 1µl well looks more like the control, than the titer of your virus solution is low. Amplify the virus one more time before you proceed with protein production.

6. The cells from the 100µl well can be harvested and lysed in Insect Lysis Buffer (Cat. No. ABP-BUF-10014). The desired protein production may be checked on Western Blot (if antibodies are available) or by SDS-PAGE gel.

Splitting cells:

Before producing the target protein on a large scale characterize gene expression from the recombinant virus, and determine the time course of protein production. The table below provides a guideline on various sizes of cell monolayers that can be used.

Type of Vessel	Cell Density*	Final Volume†
35mm dish	$0.7x10^5$	1ml
60mm dish	$2.5x10^6$	3ml
150mm dish	$2.0x10^7$	30ml
25cm² flask	$3.0x10^6$	3ml
75cm² flask	$5.0x10^6$	10ml
150cm² flask	$1.8x10^7$	20ml

*Cell density in adherent culture is approximately 50% confluent

†Final volume includes culture medium and added virus. The amount of virus to add will depend on MOI.

We strongly recommend the use of insect cell line T. ni (Cat. No. ABP-CEL-10005) for protein expression. Always include an infection with wild-type virus and a mock infection as controls. A high MOI (multiplicity of infection) is used to ensure synchronous infection and MOIs of 5, 10 and 20 should be tested. Most proteins expressed from the polyhedrin promoter reach their maximum levels somewhere between 48hr and 96hr post-infection; the best time to harvest depends on the nature of the target protein.